Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1. (Currently amended) A method of generating a double stranded (ds) recombinant nucleic acid molecule covalently linked in both strands, the method comprising contacting a first ds nucleotide sequence, at least a second ds nucleotide sequence, and at least one topoisomerase in vitro, under conditions such that the topoisomerase covalently links both strands of at least one end of the first ds nucleotide sequence to both strands of at least one end of the second ds nucleotide sequence, thereby generating a ds recombinant nucleic acid molecule covalently linked in both strands, wherein the ds recombinant nucleic acid molecule does not contain a nick in either strand at the position where the ds nucleotide sequences are joined.

2. (Original) The method of claim 1, wherein at least one of said first ds nucleotide sequence and said at least second ds nucleotide sequence comprises one of a plurality of nucleotide sequences.

3. (Original) The method of claim 2, wherein said plurality of nucleotide sequences comprises a cDNA library, a combinatorial library of nucleotide sequences, or a variegated population of nucleotide sequences.

4. (Original) The method of claim 1, wherein the topoisomerase is a site specific topoisomerase.

5. (Original) The method of claim 4, wherein the site specific topoisomerase is a type I topoisomerase.

6. (Original) The method of claim 5, wherein the topoisomerase is a type IB topoisomerase.

7. (Original) The method of claim 6, wherein the type IB topoisomerase is a poxvirus type IB topoisomerase.

8. (Original) The method of claim 1, wherein the first ds nucleotide sequence and the at least second ds nucleotide sequence each comprises a first end and a second end.

9. (Original) The method of claim 8, wherein at said first end or said second end or at both said first end and said second end, said ds first nucleotide sequence and said at least second ds nucleotide sequence comprise a topoisomerase recognition site.

10. (Original) The method of claim 9, wherein the topoisomerase recognition site is at or near the 3' terminus of said first end, said second end or both said first end and said second end of said first ds nucleotide sequence and said at least second ds nucleotide sequence.

11. (Original) The method of claim 10, wherein the topoisomerase recognition site is a recognition site for a type IB topoisomerase.

12. (Original) The method of claim 1, wherein

the first ds nucleotide sequence comprises a first end and a second end, wherein at said first end or said second end or at both said first end and said second end, said first nucleotide sequence comprises a topoisomerase recognition site at or near a 3' terminus; and

the at least a second ds nucleotide sequence comprises a first end and a second end, wherein at said first end or said second end or at both said first end and said second end, said at

least second ds nucleotide sequence comprises a topoisomerase recognition site at or near a 3' terminus.

13. (Original) The method of claim 1, wherein said first ds nucleotide sequence or said at least second ds nucleotide sequence or a combination thereof is a polymerase chain reaction (PCR) amplification product produced using a PCR primer pair, wherein at least one PCR primer of the PCR primer pair comprises a topoisomerase recognition site or a complement thereof, thereby producing an amplification product comprising a first end and a second end, wherein at said first end or said second end or both, said amplification product comprises a topoisomerase recognition site at or near the 3' terminus.

14. (Original) The method of claim 1, further comprising contacting said ds recombinant nucleic acid molecule covalently linked in both strands with an amplification primer pair; and amplifying the ds recombinant nucleic acid molecule.

15. (Currently amended) A method of generating a double stranded (ds) recombinant nucleic acid molecule covalently linked in both strands, the method comprising contacting a first topoisomerase-charged ds nucleotide sequence and at least a second topoisomerase-charged ds nucleotide sequence in vitro under conditions such that an end of a first ds nucleotide sequence having a topoisomerase covalently bound thereto contacts an end of the at least second ds nucleotide sequence having a topoisomerase covalently bound thereto, thereby generating a ds recombinant nucleic acid molecule covalently linked in both strands, wherein the ds recombinant nucleic acid molecule does not contain a nick in either strand at the position where the ds nucleotide sequences are joined.

16. (Original) The method of claim 15, comprising a first ds nucleotide sequence and a second ds nucleotide sequence.

17. (Original) The method of claim 16, wherein said first ds nucleotide sequence and said second ds nucleotide sequence are the same.

18. (Original) The method of claim 15, further comprising at least a third ds nucleotide sequence.

19. (Original) The method of claim 15, further comprising at least a third topoisomerase ds nucleotide sequence.

20. (Original) The method of claim 15, wherein said first topoisomerase-charged ds nucleotide sequence or said at least second topoisomerase-charged ds nucleotide sequence or a combination thereof is topoisomerase-charged at both ends.

21. (Original) The method of claim 15, wherein each of said first topoisomerase-charged ds nucleotide sequence and said at least second topoisomerase-charged ds nucleotide sequences comprises a topoisomerase covalently bound at one or both 3' termini.

22. (Original) The method of claim 15, wherein the topoisomerase is a type IB topoisomerase or a catalytic domain of a type IB topoisomerase.

23. (Original) The method of claim 15, wherein

a) the first topoisomerase-charged ds nucleotide sequence comprises a first end and a second end, each end comprising a 5' terminus and a 3' terminus,

wherein at said first end or said second end or both, said first topoisomerase-charged ds nucleotide sequence comprises a topoisomerase bound at the 3' terminus and a hydroxyl group at the 5' terminus of the end comprising the bound topoisomerase; and

b) the at least second topoisomerase-charged ds nucleotide sequence comprises a first end and a second end, each end comprising a 5' terminus and a 3' terminus,

wherein at said first end or said second end or both, said at least second topoisomerase-charged ds nucleotide sequence comprises a topoisomerase at the 3' terminus and a hydroxyl group at the 5' terminus of the end comprising the bound topoisomerase.

24. (Original) The method of claim 23, wherein the first topoisomerase-charged ds nucleotide sequence is topoisomerase-charged at both ends.

25. (Currently amended) A method of generating a double stranded (ds) recombinant nucleic acid molecule covalently linked in both strands, the method comprising:

a) amplifying a portion of a first ds nucleotide sequence using an amplification primer pair, wherein at least one PCR primer of the primer pair comprises a topoisomerase recognition site or a complement thereof,

thereby producing an amplified first ds nucleotide sequence comprising a first end and a second end, each end comprising a 5' terminus and a 3' terminus,

wherein at said first end or said second end or at both said first end and said second end, said amplified first ds nucleotide sequence comprises a topoisomerase recognition site at or near at least one terminus; and

b) contacting the amplified first ds nucleotide sequence; at least a second ds nucleotide sequence comprising a first end and a second end, each end comprising a 5' terminus and a 3' terminus, wherein at said first end or said second end or at both said first end and said second end, said at least second ds nucleotide sequence comprises a topoisomerase recognition site at or near at least one terminus; and at least one topoisomerase <u>in vitro</u>,

under conditions such that an end of said amplified first ds nucleotide sequence comprising a topoisomerase recognition site and an end of said at least second ds nucleotide sequence comprising a topoisomerase recognition site are contacted, thereby generating a ds recombinant nucleic acid molecule covalently linked in both strands, wherein the ds recombinant nucleic acid molecule does not contain a nick in either strand at the position where the ds nucleotide sequences are joined.

26. (Original) The method of claim 25, wherein each of the amplified first ds nucleotide sequence and the at least second ds nucleotide sequence comprises a topoisomerase recognition site at or near at least one 3' terminus.

27. (Original) The method of claim 26, wherein the at least one topoisomerase is a type IB topoisomerase.

28. (Original) The method of claim 25, wherein said amplified first ds nucleotide sequence comprises one of a plurality of nucleotide sequences.

29. (Original) The method of claim 28, wherein the plurality of nucleotide sequences is a cDNA library, a combinatorial library of nucleotide sequences, or a population of variegated nucleotide sequences.

30. (Original) The method of claim 25, wherein the amplified first ds nucleotide sequence comprises a topoisomerase recognition site at the 3' terminus at each of said first end and said second end.

31. (Original) The method of claim 25, wherein the at least a second ds nucleotide sequence comprises a linker sequence.

32. (Original) The method of claim 25, further comprising at least a third ds nucleotide sequence.

33. (Original) The method of claim 25, further comprising at least a third ds nucleotide sequence comprising a first end and a second end, each end comprising a 5' terminus and a 3' terminus, wherein said at least third ds nucleotide sequence comprises a topoisomerase recognition site at or near at least one of said 5' terminus or said 3' terminus.

34. (Original) The method of claim 33, wherein each of said amplified first ds nucleotide sequence, second ds nucleotide sequence and at least third ds nucleotide sequence comprises an overhanging sequence at an end comprising the topoisomerase recognition site.

35. (Original) The method of claim 34, wherein the topoisomerase recognition site is at or near at least one 3' terminus, and wherein the overhanging sequence is a 5' overhanging sequence.

36. (Original) The method of claim 34, wherein the overhanging sequences of ends of ds nucleotide sequences to be covalently linked are complementary.

37. (Original) The method of claim 25, wherein the at least second ds nucleotide sequence comprises or encodes a regulatory element.

38. (Original) The method of claim 37, wherein the regulatory element is a promoter, an enhancer, a silencer, a translation start site, or a polyadenylation signal.

39. (Original) The method of claim 37, wherein the regulatory element is an initiator methionine codon or a STOP codon.

40. (Original) The method of claim 25, wherein the first ds nucleotide sequence comprises an expressible nucleotide sequence.

41. (Original) The method of claim 40, wherein the expressible nucleotide sequence encodes a polypeptide.

42. (Original) The method of claim 41, wherein the expressible nucleotide sequence comprises an antisense nucleotide sequence, a tRNA, a ribozyme, an RNAi nucleotide sequence, or a triplexing nucleotide sequence.

43. (Original) The method of claim 42, wherein the tRNA is a suppressor tRNA.

44. (Original) The method of claim 25, wherein the at least second ds nucleotide sequence comprises or encodes a detectable label.

45. (Original) The method of claim 44, wherein the detectable label is an enzyme, a substrate for an enzyme, a fluorescent compound, a luminescent compound, a chemiluminescent compound, a radionuclide, a paramagnetic compound, or biotin.

46. (Original) The method of claim 25, wherein the at least second ds nucleotide sequence comprises or encodes a tag.

47. (Original) The method of claim 46, wherein the tag is an oligonucleotide tag or a peptide tag.

48. (Original) The method of claim 47, wherein the peptide tag is a polyhistidine tag, a V5 epitope, or a myc epitope.

49. (Original) The method of claim 25, wherein the at least second ds nucleotide sequence encodes a transcription activation domain or a DNA binding domain.

50. (Original) The method of claim 49, wherein the first ds nucleotide sequence comprises a plurality of nucleotide sequences.

51. (Original) The method of claim 50, wherein the plurality of nucleotide sequences is a cDNA library, a combinatorial library of nucleotide sequences, or a variegated population of nucleotide sequences.

52. (Original) The method of claim 25, wherein the first ds nucleotide sequence and at least second ds nucleotide sequence are covalently linked in a predetermined directional orientation.

53. (Original) The method of claim 25, further comprising performing a coupled transcription/translation reaction using the ds recombinant nucleic acid molecule.

54. (Original) The method of claim 25, further comprising transfecting a cell with the ds recombinant nucleic acid molecule.

55. (Original) The method of claim 25, wherein a primer of the primer pair comprises a complement of a type IB topoisomerase recognition site, said amplification primer further comprising a 5' hydroxyl group.

56. (Original) The method of claim 25, wherein the primer comprising the topoisomerase recognition site or complement thereof further comprises a nucleotide sequence of about 2 to 12 nucleotides 5' to said topoisomerase recognition site or complement thereof.

57 to 74. (Cancelled)